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Differential expression of $\beta 1$ and $\beta 2$ integrins and L-selectin on $CD4^+$ and $CD8^+$ T lymphocytes in human blood: comparative analysis between isolated cells, whole blood samples and cryopreserved preparations

M. CAVERS, B. AFZALI KHOSHKBIJARI, M. MACEY*, D. A. MCCARTHY*, S. IRSHAD & K. A. BROWN

Department of Immunobiology, The Guy's, King's College and St Thomas's Hospitals' Medical and Dental School (GKT) and

**Department of Haematology, The Royal London Hospital, London UK*

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SUMMARY

Flow cytometric analysis was used to compare the expression of adhesion molecules on human $CD4^+$ and $CD8^+$ T lymphocytes in isolated blood mononuclear cells (MNCs) in whole blood samples and in cryopreserved MNC preparations. Examination of MNCs revealed that the CD11b and CD11c components of the $\beta 2$ integrins were preferentially expressed on $CD8^+$ T cells, whereas CD62L was present on more $CD4^+$ T cells. All $CD4^+$ and $CD8^+$ T lymphocytes were positive for CD11a but the $CD8^+$ population had a higher intensity of expression of CD11a and also CD11b. Virtually identical results were obtained with T cells in whole blood samples. In relation to the $\beta 1$ integrins, the only difference between isolated $CD4^+$ and $CD8^+$ T cells was that the latter subset had a greater proportion of cells bearing CD49d. The naive cell marker CD45RA was present on the majority of $CD8^+$ T cells whereas CD45RA and the memory marker CD45RO were evenly distributed within the $CD4^+$ T cell subset. Although cryopreservation of lymphocytes did not modify the expression of $\beta 1$ and $\beta 2$ integrins it produced a marked reduction in the percentage of $CD4^+$ and $CD8^+$ T cells bearing CD62L. With regard to endothelial interactions, it appears that cryopreserved lymphocytes are suitable for inclusion in studies of integrin-mediated adhesion but not for those relating to tethering or recognition of addressins on high endothelial venules. Differences in adhesion molecule expression between $CD4^+$ and $CD8^+$ T lymphocytes could underlie the selective extravasation of these subsets into sites of infection and inflammation.

Keywords adhesion molecules flow cytometry human T lymphocytes

INTRODUCTION

Surface adhesion molecules on T lymphocytes control extravasation into lymphoid and non-lymphoid tissue [1,2]. Lymphocyte attachment to blood vessel walls is governed in part by the $\beta 1$ integrins (CD49/CD29 family) recognizing vascular adhesion molecule-1 (VCAM-1) and components of the extracellular matrix and by members of the $\beta 2$ integrins (CD11/CD18 family) binding to intercellular adhesion molecule-1 (ICAM-1). The initial tethering of T lymphocytes to endothelial cells, which precedes the firm adhesion mediated by the integrins, is dependent upon the function of CD62L [2], which is a receptor that interacts with addressins on high endothelial venules and is responsible for the 'homing' to secondary lymphoid tissue of circulating lymphocytes [3]. Adhesion molecules also facilitate the engagement of T lymphocytes with antigen-presenting cells. This binding allows

the T cell receptor to scan the surface of the antigen-presenting cell for antigen and to sustain cell contact, should antigen recognition occur, in order to initiate and complete effector mechanisms.

Quantitative or qualitative variations in surface adhesion molecules are likely to contribute to the selective extravasation of distinct classes of leucocytes. Most lymphocytes express CD49d/CD29 and very few CD11b, in contrast to neutrophils, which are virtually all positive for CD11b but negative for CD49d [4,5]. Differences in adhesion molecule expression may be responsible for the preferential sequestration of $CD4^+$ T cells and $CD8^+$ T cells in lymph nodes and Peyer's patches, respectively [6], to the selective migration of $CD4^+$ T cells to sites of delayed hypersensitivity and chronic inflammatory lesions [7] and to the distinct functional roles of $CD4^+$ and $CD8^+$ T cells. To investigate whether these subsets exhibit characteristic adhesion molecule profiles, we compared the distribution and level of expression of CD11a, CD11b, CD11c, CD49d, CD29 and L-selectin on circulating human peripheral blood $CD4^+$ and $CD8^+$ T

Correspondence: Dr K A Brown, Department of Immunobiology, 3rd Floor New Guy's House, Guy's Hospital, London SE1 9RT, UK.

E-mail: alun.brown@kcl.ac.uk

cells in whole blood samples and in mononuclear leucocytes isolated from normal healthy subjects. The phenotypic and functional assessment of lymphocytes from patients with various disorders are occasionally hampered by the late arrival of blood samples to the laboratory. Cryopreservation of the cells overcomes this problem and this procedure is also advantageous to lymphocyte studies in longitudinal investigations. Accordingly, another feature of the present study was to ascertain whether the process of cryopreservation and thawing modified the expression of adhesion molecules on CD4⁺ and CD8⁺ T lymphocytes.

MATERIALS AND METHODS

Antibodies and reagents

Hanks's Balanced Salt Solution (HBSS, without phenol red), HEPES buffer, bovine serum albumin (BSA), fetal bovine serum (FBS), dimethyl sulphoxide (DMSO) and Histopaque-1077 were all obtained from Sigma Chemical Co, Irvine, UK. Fluorescein isothiocyanate (FITC)-conjugated antibodies against CD11a (25-3), CD11b (Bear-1), CD29 (K20), CD49d (HP2/1), CD62L (DREG56), CD4 (13B8-2), CD8 (B9-11), phycoerythrin-cyanine 5-1 (PC5)-conjugated antibody against CD3 (UCHT1) and phycoerythrin (PE)-conjugated antibodies against CD4 (13B8-2), CD8 (B9-11) and CD11c (BU15) were purchased from Immunotech (Marseille, France). Other FITC-conjugated antibodies to CD49b (AK7), CD49e (SAM1), CD49f (450-30 A), CD45RA (F8-11-13) and CD45RO (UCHL1) were obtained from Serotec (UK). Isotype matched conjugated negative control antibodies (IgG1, IgG2a and IgG2b) were purchased from each of the above manufacturers. Red cell lysing solution, 'FACSlyse' (Becton Dickinson) was diluted 1 in 10 (v/v) from stock with distilled water and the fluorescent nuclear dye LDS-751 (Molecular Probes, Leiden, the Netherlands) was diluted 1 in 100 (v/v) from a stock solution of 0.02% (v/v) in methanol using a 0.5% (v/v) solution of formaldehyde in HBSS as a diluent.

Processing of blood samples

Blood samples were obtained from 38 normal human volunteers. Experiments were performed on either fresh whole blood (six females, six males, age range 21–52 years) or on isolated mononuclear cells (17 females, nine males, age range 20–63 years). For the whole blood analysis, 4 ml blood were collected in K₂ EDTA tubes (Becton-Dickinson). For analysis of isolated mononuclear cells (MNCs), 30 ml venous blood from each subject were collected in lithium heparin tubes (Becton-Dickinson), diluted 1:1 (v/v) with warmed saline solution, layered onto Histopaque-1077 and centrifuged at 800 *g* for 20 min. The MNC layer was washed twice in HBSS with 1% HEPES and aliquots prepared for immediate flow cytometric analysis or cryopreserved for later study.

Cryopreservation of mononuclear cells

MNCs, 7×10^6 , were suspended in 1 ml 90% heat inactivated FBS with 10% DMSO and stored in an insulated container at -80°C for 24–48 h before introduction into liquid nitrogen. Cells were thawed rapidly on the day of analysis, washed in 20 ml cold HEPES-buffered HBSS and resuspended in HBSS, 1% HEPES buffer, 0.5% BSA (labelling solution) at a concentration of $1.25 \times 10^6/\text{ml}$.

Labelling of lymphocytes with antibodies

Whole blood samples. For each analysis 25 μl anticoagulated whole blood were labelled with 5 μl of antibody against either CD4 or CD8 and with 5 μl of an antibody against one of the adhesion molecules under investigation. Each sample was incubated for 10 min on ice in the dark followed by the addition of a further 75 μl HBSS. Red cells were lysed by adding 2 ml FACSlyse to each sample for 10 min in the dark at room temperature. Samples were centrifuged at 300 *g* for 5 min, the supernatant removed, the pellet resuspended in 1 ml HBSS and further centrifuged at 200 *g* for 5 min. The supernatant was aspirated and 0.5 ml LDS nuclear stain solution added to each sample. At the same time, positive and negative single antibody controls were set up with 5 μl of PE-conjugated antibody against CD4 and CD8, 5 μl of FITC-conjugated antibody against each of the adhesion molecules tested, or 5 μl of isotype-matched PE- and FITC-conjugated mouse IgG1 antibodies.

Isolated cells. For each test, 2.5×10^5 MNCs in 200 μl labelling solution (see Cryopreservation of mononuclear cells) were labelled with 5 μl PC5-conjugated antibody against CD3, 10 μl FITC- or PE-conjugated antibody against CD4 or CD8 and 5 μl or 10 μl (as appropriate) FITC- or PE-conjugated antibody against the adhesion molecules or CD45. At the same time triple-negative controls of mouse IgG1 PE-IgG1 PC5- and either mouse IgG1, IgG2a or IgG2b FITC-conjugated antibodies were prepared. Single positive controls were comprised of the PC5-conjugated antibody against CD3, the PE-conjugated antibody against CD8 and FITC-conjugated antibodies to each adhesion molecule. A further negative control of unlabelled cells was also used for each experiment. Each of the above preparations was incubated for 20 min in the dark at room temperature. The cells were then washed in 1 ml HEPES-buffered HBSS by centrifugation at 600 *g* for 5 min and resuspended in 400 μl labelling solution.

Flow cytometry

Whole blood samples underwent two-colour analysis on a Becton Dickinson FACScan flow cytometer using the Consort 32 Lysis II program. Lymphocytes were identified by their position on a dot plot of light forward *versus* side scatter as well as side scatter *versus* LDS-751 staining intensity (monitored in the FL3 channel). Three-colour analysis was undertaken on the isolated MNCs using a Coulter EPICS MCL XL2 flow cytometer and gating the lymphocyte population in the plot of forward *versus* side scatter. For each sample, 5000 events were recorded. By including the antibody to CD3 (monitored in the FL4 channel) and gating on the CD8/CD3 double positive lymphocytes within the FL2 *versus* FL4 plot, the CD8⁺CD3⁺ natural killer cell population, which may appear within the lymphocyte gate, was excluded from analysis. Adhesion molecule expression was recorded as percentage of lymphocytes and mean fluorescence intensity as monitored in the double-positive quadrant of the FL1 *versus* FL2 plot gated for CD3⁺ lymphocytes (see Fig. 1).

Statistical analysis

The data were normally distributed. Hence, comparisons between CD4 and CD8 lymphocyte expression, as well as between cryopreserved and non-cryopreserved cells, were performed using Student's paired *t*-test. *P*-values < 0.05 were set as the level of significance.

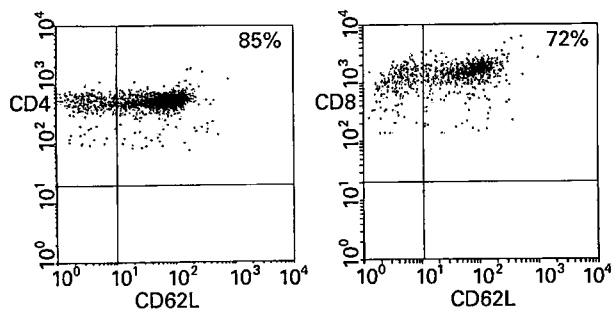


Fig. 1. Flow cytometric profile of $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes from an isolated population of MNCs, expressing the adhesion molecule CD62L.

RESULTS

Distribution of integrins and CD62L on $CD4^+$ and $CD8^+$ T lymphocytes in whole blood samples and isolated mononuclear cells

The expression of CD11a, CD11b, CD11c and CD62L was examined on $CD4^+$ and $CD8^+$ T lymphocytes in whole blood samples obtained from 14 healthy subjects. Figure 2a shows that

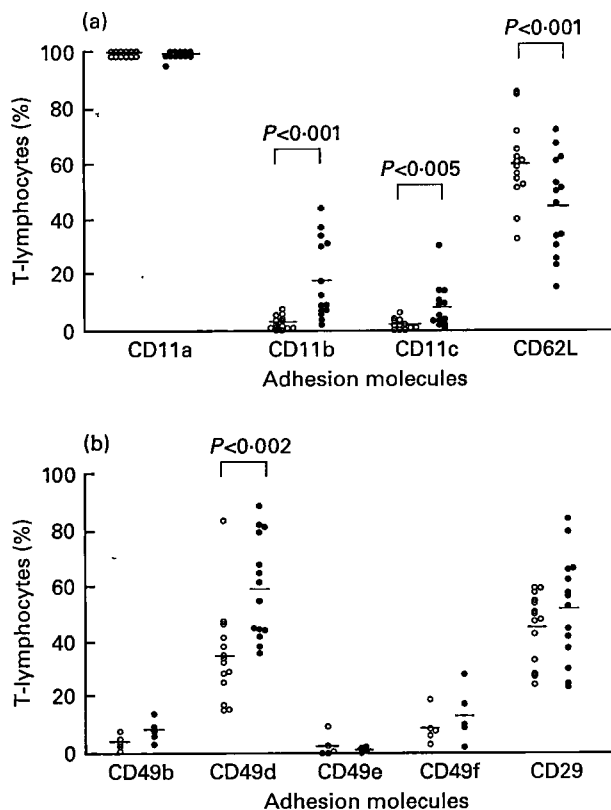


Fig. 2. Within mononuclear cell isolates $CD8^+$ T cells preferentially express CD11b, CD11c and CD49d in relation to $CD4^+$ T cells but less CD62L. Blood mononuclear cells were isolated from 14 subjects and examined for their distribution of (a) CD11a, CD11b, CD11c and CD62L and (b) CD49b, CD49d, CD49e, CD49f and CD29. Results are presented as the percentage of positively stained $CD4^+$ and $CD8^+$ T lymphocytes. Horizontal lines represent mean values. Differences (P -values) between $CD4^+$ and $CD8^+$ T cells in the distribution of adhesion molecules are indicated on the figure. $\circ$, $CD4^+$; $\bullet$, $CD8^+$.

$CD11b$ was expressed on a higher percentage of $CD8^+$ T cells (mean $18 \pm 14\%$) than $CD4^+$ T cells (mean $2 \pm 2\%$; $P < 0.001$) and that $CD11c$ was preferentially associated with $CD8^+$ T cells (mean $7 \pm 8\%$ versus mean $2 \pm 2\%$ for $CD4^+$ T cells; $P < 0.005$). In contrast, the distribution of lymphocytes bearing CD62L was greater in the $CD4^+$ subset (mean $60 \pm 14\%$) than in the $CD8^+$ population (mean $44 \pm 18\%$; $P < 0.001$). Virtually all $CD4^+$ and $CD8^+$ T cells displayed CD11a on their surface with the $CD8^+$ subset (Table 1) exhibiting the higher intensity of expression of the molecules (mean MFI 105 ± 41 versus 75 ± 17 ; $P < 0.05$). Of the other adhesion molecules, Table 1 further shows that relative to $CD4^+$ cells, the $CD8^+$ T cells had a higher MFI for CD11b ($P < 0.01$) and a similar expression of CD11c and CD62L. The results show that differences in the distribution of CD11b, CD11c and CD62L on $CD4^+$ and $CD8^+$ T cells in whole blood were retained upon isolation of the MNCs. For each adhesion molecule investigated the level of distribution (i.e. % stained cells) and MFI were remarkably similar on $CD4^+$ and $CD8^+$ T cells in whole blood samples and in isolated MNC preparations.

Since adhesion molecules may be up-regulated by isolation [8], the studies were extended to the measurement of the above adhesion molecules on $CD4^+$ and $CD8^+$ T lymphocytes in whole blood samples from an additional 12 subjects. In comparison with the $CD4^+$ population there were more $CD8^+$ T cells expressing CD11b (mean $25 \pm 16\%$ versus $4 \pm 7\%$; $P < 0.001$) and CD11c (mean $8 \pm 8\%$ versus $3 \pm 1\%$; $P < 0.005$) but fewer cells bearing CD62L (mean $56 \pm 14\%$ versus $75 \pm 12\%$; $P < 0.005$). Although CD11a was present on all lymphocytes, in comparison with $CD4^+$ T cells the mean fluorescence intensity (MFI) of this integrin and CD11b was higher on $CD8^+$ T cells ($P < 0.01$). There was no difference between $CD4^+$ and $CD8^+$ T cells in their MFI values for CD11c and CD62L.

The next stage of the study assessed the expression of $\beta 1$ integrins on $CD4^+$ and $CD8^+$ T cells in MNC preparations. Figure 2b shows that CD49d was more prevalent on $CD8^+$ T cells (mean $60 \pm 18\%$) than $CD4^+$ T cells (mean $35 \pm 17\%$; $P < 0.002$), but that CD29, the β chain of this subfamily, was equally distributed on $CD8^+$ T cells (mean 53%) and $CD4^+$ T cells (mean 47%). Very few lymphocytes expressed CD49b, CD49e and CD49f and the intensity of expression for all of the $\beta 1$ integrins was similar for both T cell subsets (Table 1).

Cryopreservation and the expression of adhesion molecules

Mononuclear leucocytes were isolated from seven healthy subjects and aliquots of the cells were immediately analysed for their expression of CD11a, CD11b, CD11c, CD49d, CD29 and CD62L, whereas the remainder were cryopreserved. The stored cells were thawed approximately 1 month later and examined for their expression of the above adhesion molecules. Figure 3a shows that with the fresh preparations of MNCs, the proportion of cells bearing CD11b, CD11c and CD49d was higher in the $CD4^+$ T cell subset. Similar results were obtained with the cryopreserved MNCs (Fig. 3b). However, a major distinction between the fresh and stored cells was in their distribution of CD62L. With the non-cryopreserved cells (Fig. 3a), CD62L was present on the majority of $CD4^+$ T cells (mean $61 \pm 19\%$) in comparison to a mean $40 \pm 22\%$ ($P < 0.002$) of the $CD8^+$ T cells. This difference was not apparent within the cryopreserved cells (Fig. 3b) because CD62L was only expressed on a small minority ($P < 0.001$) of $CD8^+$ T cells (mean $12 \pm 15\%$) and $CD4^+$ T cells (mean

Table 1. Intensity of expression of $\beta 2$, $\beta 1$ integrins and L-selectin on $CD4^+$ and $CD8^+$ T cells in isolated blood mononuclear leucocytes

	Number of subjects	MFI					
		$\beta 2$ integrins			Number of subjects	$\beta 1$ integrins	
		$CD4^+$	$CD8^+$			$CD4^+$	$CD8^+$
CD11a	(14)	75 $\pm$ 17	105 $\pm$ 41*	CD49b	(5)	15 $\pm$ 2	17 $\pm$ 5
CD11b	(14)	22 $\pm$ 3	33 $\pm$ 6**	CD49d	(14)	25 $\pm$ 6	23 $\pm$ 4
CD11c	(14)	39 $\pm$ 20	43 $\pm$ 16	CD49e	(5)	18 $\pm$ 7	22 $\pm$ 21
CD62L	(14)	35 $\pm$ 13	37 $\pm$ 14	CD49f	(5)	15 $\pm$ 1	18 $\pm$ 3
				CD29	(14)	34 $\pm$ 7	31 $\pm$ 6

The intensity of expression of adhesion molecules is presented in terms of the mean fluorescence intensity (MFI). Results represent the mean values $\pm$ standard deviation of mononuclear leucocytes prepared from five or 14 healthy subjects. * $P < 0.05$, ** $P < 0.01$ compared with expression on $CD4^+$ T cells.

15 $\pm$ 18%). From Table 2 it is apparent that the MFI of the adhesion molecules investigated was not modified by cryopreservation and thawing.

The last phase of the study investigated the distribution of CD45RO and CD45RA antigens on $CD4^+$ and $CD8^+$ T lymphocytes in isolated MNCs prior to and after cryopreservation.

Examination of freshly isolated MNCs from five healthy subjects revealed a trend for more of the $CD8^+$ T cells to express CD45RA (mean 77 $\pm$ 7%) than the $CD4^+$ T cells (mean 61 $\pm$ 12%), whereas approximately just under 50% of the $CD4^+$ T cells and $CD8^+$ T cells expressed CD45RO. Similar distributions in the CD45RA and CD45RO subsets were apparent in the MNCs following cryopreservation except that the percentage of $CD8^+$ T cells bearing CD45RA (mean 79 $\pm$ 10%) was greater than the $CD4^+$ T cells (mean 65 $\pm$ 9%; $P < 0.002$).

DISCUSSION

This study demonstrates that human blood T lymphocytes expressing CD11b, CD11c and CD49d are more prevalent within the $CD8^+$ population than the $CD4^+$ population, although the $CD8^+$ subset contained fewer cells bearing CD62L. In addition, the $CD8^+$ T cells had a higher level of expression of CD11a and CD11b. Comparable results were obtained with preparations of

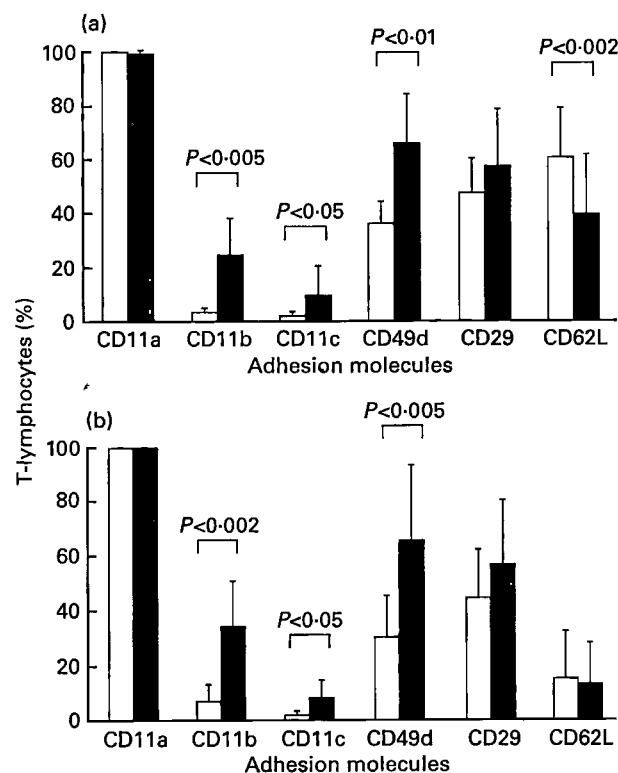


Fig. 3. Adhesion molecule expression on $CD4^+$ and $CD8^+$ cryopreserved T lymphocytes. The expression of CD11a, CD11b, CD11c, CD49d, CD29 and CD62L was measured on mononuclear cells isolated from the blood of seven healthy subjects (a) prior to cryopreservation and (b) after cryopreservation. Results are expressed as the mean percentage of $CD4^+$ and $CD8^+$ T lymphocytes bearing the adhesion molecules. Differences (P -values) between $CD4^+$ and $CD8^+$ T cells in the distribution of adhesion molecules are indicated on the figure. $\square$, $CD4^+$; $\blacksquare$, $CD8^+$.

Table 2. Intensity of expression of adhesion molecules on cryopreserved $CD4^+$ and $CD8^+$ T cells

Adhesion molecule	MFI			
	Non-cryopreserved		Cryopreserved	
	$CD4^+$	$CD8^+$	$CD4^+$	$CD8^+$
CD11a	84 $\pm$ 20	128 $\pm$ 44*	69 $\pm$ 21	125 $\pm$ 48*
CD11b	22 $\pm$ 3	34 $\pm$ 8**	23 $\pm$ 3	31 $\pm$ 8**
CD11c	26 $\pm$ 10	37 $\pm$ 15	87 $\pm$ 86	61 $\pm$ 63
CD49d	26 $\pm$ 7	24 $\pm$ 4	23 $\pm$ 5	22 $\pm$ 3
CD29	36 $\pm$ 5	31 $\pm$ 3	33 $\pm$ 7	31 $\pm$ 8
CD62L	38 $\pm$ 17	37 $\pm$ 18	35 $\pm$ 12	26 $\pm$ 15

The intensity of expression of adhesion molecules is presented in terms of the mean fluorescence intensity (MFI). Results represent the mean values $\pm$ standard deviation of mononuclear leucocyte preparations from seven healthy subjects. Lymphocytes were analysed for adhesion molecule expression immediately upon isolation (non-cryopreserved) or after approximately 1 month of cryopreservation. * $P < 0.05$ compared with $CD4^+$ cells. ** $P < 0.02$ compared with $CD4^+$ cells.

isolated mononuclear leucocytes and whole blood samples. Differences between the expression of adhesion molecules on CD4⁺ and CD8⁺ T cells were maintained upon cryopreservation except for CD62L whose distribution was significantly reduced in both subsets.

Despite the known confinement of CD11b and CD11c to a minority of T lymphocytes [9–11], we noted that in relation to CD4⁺ T cells, more CD8⁺ T cells had CD11b and CD11c on their surface and a higher expression of CD11b. The CD8⁺ cells that bear CD11b suppress T cell proliferation [12] and CD11c, which is expressed by some cytotoxic T cell clones, enhances cytotoxicity at the level of conjugate formation between effector and target cells [13]. The binding of cytotoxic T cells to epithelial cells and fibroblasts [5,14] is augmented by CD11a, which also promotes lymphocyte attachment to endothelium by recognition of ICAM-1 and ICAM-2 [2]. CD11a was present on all CD4⁺ and CD8⁺ T lymphocytes and its level of expression was greater on CD8⁺ T cells, an observation that supports previous reports [15–17].

Of the β 1 integrins, CD49d is expressed on resting lymphocytes whereas most of the other members of this family are restricted to activated cells [4]. This molecule recognizes VCAM-1, which is induced on cytokine-activated endothelial cells, and the extracellular matrix protein, fibronectin [4]. The observation that CD49d was present on a higher proportion of CD8⁺ T cells than CD4⁺ T cells implies that cytotoxic T cells are favourably disposed towards binding to endothelium stimulated by cytokines and to the extracellular matrix of damaged vessels. That CD49b and CD49e were only expressed on a very small percentage of CD4⁺ and CD8⁺ T cells is in accord with the low number of activated T cells within the vasculature of healthy subjects.

CD62L, which is present on the surface of most leucocytes [2], promotes tethering to inflamed vascular endothelium and controls lymphocyte homing to secondary lymphoid tissue by interacting with addressins such as MadCAM-1 expressed on high endothelial venules [3]. The preferential expression of CD62L on CD4⁺ T cells in comparison with CD8⁺ T cells could contribute to the selective accumulation of CD4⁺ T cells in chronic inflammatory lesions characterized by HEV, such as the rheumatoid joint [18], and to the entry of CD4⁺ T cells into early MS lesions and CD8⁺ T cells into late lesions [19]. An earlier report of the preferential association of CD62L with CD4⁺ T cells rather than CD8⁺ T cells [20] may have arisen mistakenly from the inclusion of CD8⁺ NK cells in the mononuclear cell population [12]. To exclude NK cells from the current study, all measurements on MNCs were undertaken on CD4⁺ and CD8⁺ lymphocytes that also expressed CD3. Following cell activation CD62L is cleaved from the surface [21,22] and this effect may have contributed to its decreased distribution and low level of expression on CD4⁺ and CD8⁺ T lymphocytes during the thawing of cryopreserved cells. Additional studies by our group show that cryopreservation does not alter the adhesive properties of T lymphocytes for cultured endothelium in a static adherence assay [23] and for blood vessel walls exposed in tissue sections of normal brain. However, the same caveat is unlikely to apply to the 'tethering' of T lymphocytes to endothelial cells and to recognition of addressins on high endothelial venules, since each of these events is dependent upon CD62L expression.

In the present study the CD8⁺ T cells were comprised mainly of naive cells, whereas similar ratios of naive and memory cells were present in the CD4⁺ population, both findings agreeing with

earlier observations [17]. Naive lymphocytes (CD45RA⁺), which have yet to encounter antigen, recirculate from blood to secondary lymphoid tissue whereas circulating memory cells (CD45RO⁺), that have undergone antigen-induced clonal proliferation, preferentially enter non-lymphoid tissue [24]. Such differences in migratory behaviour are associated in part with a greater proportion of the CD45RA⁺ cells bearing CD62L, which is compatible with the present finding, and with CD45RO⁺ cells generally possessing a higher expression of β 1 and β 2 integrins than CD45RA⁺ cells [1,24]. Most of the LFA-1 on naive T cells is considered to be in an inactive form, whereas memory cells predominantly express active molecules [25]. On this basis we would have anticipated the CD8⁺ T cells possess a lower surface expression of integrins compared with CD4⁺ T cells rather than the converse, which was a greater distribution of CD11b, CD11c and CD49d and a higher expression of CD11a and CD11b. Moreover, our recent work reveals that an antibody directed against the active form of CD11a stains CD8⁺ T cells in preference to CD4⁺ T cells (unpublished observations). Thus, the current findings demonstrate that the level of CD11a expression and activation is higher on CD8⁺ T cells than CD4⁺ T cells, most of which express the phenotype of naive cells.

Although an up-regulation of CD11b, CD11c and CD49d on cytotoxic CD8⁺ T cells may increase their predisposition to adhere to target cells and to endothelial cells, the latter consideration is questioned by binding studies which show that CD4⁺ T cells are often more adherent than CD8⁺ T cells to endothelial monolayers in static assays [26,27] and to blood vessel walls exposed in tissue sections [28]. With respect to the latter assay, CD62L could be contributing to adhesion since the glass slides bearing tissue sections overlaid with lymphocytes are constantly rotated during an incubation period which is of shorter duration than the endothelial binding assay. Furthermore, the preferential attachment of CD4⁺ T lymphocytes to endothelium could be due to subtle variations in the degree of activation of adhesion molecules, other than CD11a, on the surface of CD4⁺ and CD8⁺ T cells or to differences in the expression and/or activity of adhesion-promoting determinants that were not investigated in this study. Understanding the distribution and intensity of expression of adhesion molecules on lymphocytes in whole blood samples and in leucocyte isolates from healthy subjects should benefit their study in several immune and inflammatory disorders. However, in evaluating the contribution of these molecules to blood vessel wall interactions, caution needs to be exercised regarding the suitability of some established adhesion assays for monitoring the binding properties of cryopreserved lymphocytes.

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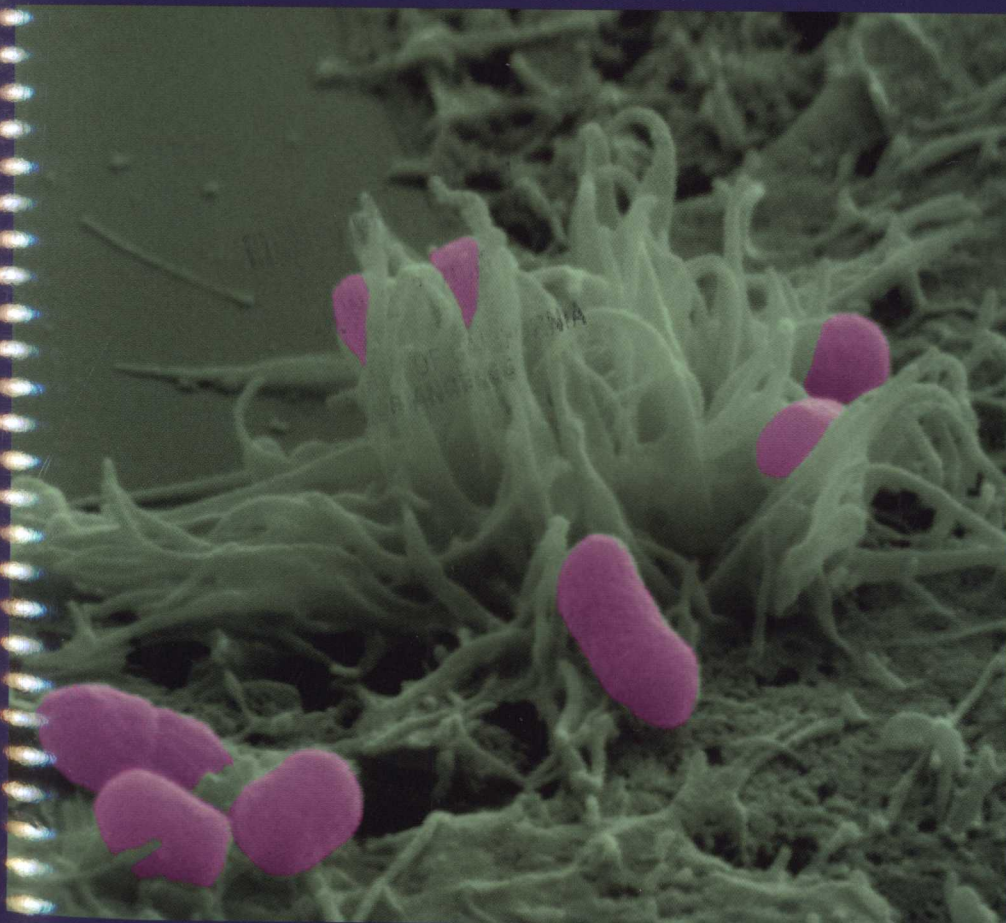
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Editorial correspondence. Editorial correspondence should be directed to Sharon Tobin, *Clinical and Experimental Immunology*, British Society for Immunology, Triangle House, Broomhill Road, London SW18 4HX, UK (Tel: +44 (0)20 8875 2441; Fax: +44 (0)20 8875 2422; Email: cei@immunology.org).

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Corrigenda

In [1], one of the author names was cited incorrectly: 'B. Afzali Khoshkbijari' should have been cited as 'B. Afzali'.

In [2], in the section 'Short-term prophylaxis', the following sentence was incorrect: 'However, it seems that most authors prefer attenuated androgens, where concentrate is not used, even in children [18,54] at a dose of 100–600 mg/day for danazol or 2–6 mg/day for stanozolol, given 48 h before and after surgery [1,18,46,54]'. It should have read 'However, it seems that most authors prefer attenuated androgens, where concentrate is not used, even in children [18,54] at a dose of 100–600 mg/day for danazol or 2–6 mg/day for stanozolol, given 5 days before and 48 h after surgery [1,18,46,54]'. In [2], Table 3 was similarly incorrect. The corrected table is printed below.

Table 3. Treatment summary. The regimen for each affected individual should be guided by the severity of their disease and thus titred to individual need. The following is a guide to the dosage and summarizes the advice given in the text.

Intervention	Therapy	Dosage (adult)	Dosage (children)	Monitoring tests
Long-term prophylaxis	Attenuated androgens	Danazol 200 mg once or twice per day; up to 400 mg/day in <20% of cases Stanozolol up to 5 mg once or twice per day Oxandrolone 2.5–20 mg divided dose 2–4 times per day (use lowest effective maintenance dose, consider alternate-day or 2× weekly regimen)	[Only if indicated (very rare), see text] Danazol 100–200 mg/day (use lowest effective maintenance dose, consider alternate-day or 2× weekly regimen)	Six-monthly: liver function tests Annual: lipid profile Biennial: hepatic ultrasound (annual after 10 years' treatment)
	Tranexamic acid	Starting dose 1–1.5 g 2–3 times per day, reducing to 0.5 g once or twice per day	1–2 g per day; dosage depends on age and size; general guide is 50 mg/kg/day (use lowest effective maintenance dose, consider alternate-day or 2× weekly regimen)	Six-monthly: liver function tests
Short-term prophylaxis (e.g. for dental work)	C1 inhibitor concentrate	500–1500 U up to 24 h prior to procedure	<10 years old 500 U, >10 years old 1000 U up to 24 h prior to procedure	
	Attenuated androgens	Danazol 100–600 mg/day for 5 days before and 48 h after procedure Stanozolol 2–6 mg/day for 5 days before and 48 h after procedure	Danazol 300 mg/day for 5 days before and 48 h after procedure	
	Tranexamic acid	1 g given four times daily for 48 h before and after procedure	500 mg given four times daily for 48 h before and after procedure	
Emergency care for acute attacks	C1 inhibitor concentrate	500–1500 U; additional infusion and reassessment if symptoms persist for >2 h	<10 years old 500 U, >10 years old 1000 U	Baseline: liver function tests, hepatitis virology
	Attenuated androgens	Danazol up to 1 g/day Stanozolol up to 16 mg/day		
	Tranexamic acid	1 g given four times daily for 48 h		
	Fresh frozen plasma	2 units (only for use where C1 INH concentrate not available)		Baseline: liver function tests, hepatitis virology
Pregnancy	Pain relief	As appropriate		
	Attenuated androgens	Contraindicated		
	Tranexamic acid	May be used with caution		
	C1 inhibitor concentrate	Emergency care as above. Severe cases may require regular replacement		

Corrigenda

We apologize for these errors.

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- 1 Cavers M, Afzali Khoshkijari B, Macey M, McCarthy A, Irshad S, Brown KA. Differential expression of $\beta 1$ and $\beta 2$ integrins and 1-selectin on CD4⁺ and CD8⁺ T lymphocytes in human blood: comparative analysis between isolated cells, whole blood samples and cryopreserved preparations. *Clin Exp Immunol* 2002; **127**:60–5.
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